Reexamination of Sexual Dimorphism and Female Reproduction in the Many-Lined Sun Skink *Eutropis multifasciata* from China

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Abstract We reexamined sexual dimorphism and female reproduction in the Many-Lined Sun Skink *Eutropis multifasciata* from Hainan, China. Our data confirm that adults are sexually dimorphic in body size and shape, with males being the larger sex and larger in head size but shorter in abdomen length than females of the same snout-vent length (SVL). The rate at which head width increased with SVL was greater in males as opposed to the previous conclusion that the rate does not differ between the sexes. Maternal size was the main determinant of reproductive investment, with larger females generally producing more, as well as larger, offspring. Females produced up to nine offspring per litter as opposed to the previously reported 2–7. Most females gave birth between March and August, a time period approximately four months longer than that (May–June) reported previously. Females with a higher fecundity tended to produce smaller offspring as opposed to the previous conclusion that females do not tradeoff offspring size against number. Litter size, neonate mass and litter mass remained remarkably constant among years, and litter mass was more tightly related to female body size than litter size or neonate mass. Smaller females could produce relatively heavier litters without a concomitant reduction in postpartum body condition.

Keywords Scincidae, *Eutropis multifasciata*, sexual dimorphism, litter size, neonate size, reproductive output, offspring size-number trade-off

1. Introduction

Skinks formerly within the pantropical genus *Mabuya* were recently assigned into four genera, and only the South and Central American species retain that generic designation (Mausfeld *et al*., 2002). The other three genera are *Chioninia* endemic to Cape Verde Islands located in the central Atlantic Ocean, *Euprepis* in Africa, and *Eutropis* in Asia (Mausfeld *et al*., 2002). All species within the American genus are viviparous with various degrees of placentotrophic fetal nutrition, whereas the African and Asian genera possess both oviparous and viviparous species (Fitch, 1970; Shine, 1985; Blackburn and Vitt, 1992; Mausfeld *et al*., 2002; Ramírez-Pinilla, 2006). Although more than 100 species of "*Mabuya*"

skinks have been described worldwide, most of our knowledge about this group of animals is based on a few species. A majority of studies have been conducted in America and Africa, although some biological and ecological aspects of two Asian forms, the Long-Tailed Sun Skink *Eutropis* (*Mabuya*) *longicaudata* (Huang, 1994, 1995, 2006a, b, 2007; Sun *et al*., 2012a) and the Many-Lined Sun Skink *Eut. multifasciata* (Ji *et al*., 2006, 2007; Lin *et al*., 2008; Li *et al*., 2010; Sun *et al*., 2009, 2012a, b), have been described. Moreover, previous studies on "*Mabuya*" skinks have focused heavily on placental morphogenesis and structure in viviparous species, largely because both lecithotrophic fetal nutrition (the majority of nutrients for embryonic development are provided by the yolk-rich ovum) and advanced placentotrophic fetal nutrition (nutrients are transferred from mother to embryo via the placenta) have been observed in this group of animals (Blackburn and Vitt, 1992; Ramírez-Pinilla, 2006; Blackburn and Flemming,

2010).

Male and/or female reproductive cycles have been described in detail only for five viviparous species, the Cape Three-Lined Skink *Euprepis* (*Mabuya*) *capensis* (Flemming, 1994), the African Striped Skink *Eup. striata* (Patterson, 1990), *Eut. multifasciata* (Sun *et al.*, 2012b), the Cope's Mabuya *Mabuya frenata* (Vrcibradic and Rocha, 1998), and the Slippery-Backed Mabuya *M. mabouya* (Ramírez-Pinilla *et al.*, 2002). Males and females are seasonally reproductive in *Eup. capensis* (Flemming, 1994), *Eut. multifasciata* (Sun *et al.*, 2012b), and *M. frenata* (Vrcibradic and Rocha, 1998), but reproduce throughout the year in *M. mabouya* (Ramírez-Pinilla *et al.*, 2002). In two subspecies of *Eup. striata*, females of the low altitude subspecies (*Eup. s. striata*) are non-seasonal breeders, whereas females of the high altitude subspecies (*Eup. s. punctatissima*) are seasonal breeders that give birth in the late dry season only (Patterson, 1990). Previous studies of "*Mabuya*" skinks generally show that environmental factors, especially temperature and moisture, may influence reproductive activity in both sexes (Sun *et al.*, 2012b).

There are four "*Mabuya*" skinks in China (Ota *et al.*, 1993; Huang, 1998; Ota and Huang, 2000), two (*Eut. longicaudata* and *Eut. multifasciata*) of which occur in two island provinces (Hainan and Taiwan) and three mainland provinces (Yunnan, Guangxi and Guangdong). The other two, the Cuming's Mabuya *Eut. cumingi* and the Many-Keeled Mabuya *Eut. multicarinata*, can be found only on Lanyu Island, Taiwan, China. *Eutropis longicaudata* and *Eut. multifasciata* coexist in several areas of China but differ in reproductive mode, with fecundity (clutch or litter size) being higher in the oviparous form (*Eut. longicaudata*) (Huang, 1994; Ji *et al.*, 2006; Sun *et al.*, 2012a). Previous studies on these two species support the idea that life-history strategies and reproductive patterns of "*Mabuya*" skinks are diverse, even within a particular geographical or climatic zone (Vitt and Blackburn, 1983).

Sexual dimorphism and female reproduction of *Eut. multifasciata* have been examined in earlier studies conducted between 2002 and 2008, using individuals from an island population in Hainan, southern China (Ji *et al.*, 2006, 2007; Sun *et al.*, 2012a). Main conclusions drawn in these studies include: 1) males are the larger sex and have larger heads than females of the same snout-vent length (SVL); 2) females in nature often produce a single litter of 2−7 offspring per breeding season between mid-May and mid-June, and they do not tradeoff offspring size against number; 3) fecundity (litter size),

offspring size (neonate mass) and reproductive output (litter mass) are positively related to female SVL, and gestation temperature affects morphology and locomotor performance but not overall body size or mass of offspring; and 4) the detrimental effects of extreme ambient temperatures on offspring phenotype can be well buffered through maternal thermoregulation. Here, we present the data collected between 2009 and 2011 in a project addressing climatic correlates of reproductive activities and hormonally regulated reproduction. We address the following four questions: 1) How similar are the results of the present study to those reported previously? 2) Can the data collected more recently tell us something not found previously? 3) Do litter size, neonate mass and litter mass vary among years? 4) How tightly are these three reproductive variables related to female size?

2. Materials and Methods

A total of 2250 individuals were collected from the previously studied population located between Lingshui (18°48′ N, 110°01′ E) and Ledong (18°44′ N, 109°10′ E), Hainan from March 2009 to September 2011. Most individuals were released at the site of capture following the collection of morphological data. Each individual was measured for snout-vent length (SVL). Of the 2183 (1615 females and 568 males) individuals (> 86 mm SVL) considered sexually mature, 767 adults (500 females and 267 males) were also measured for head length (HL, from the snout to the anterior edge of tympanum), head width (HW, taken at the posterior end of the mandible), and abdomen length (AL, the distance between the points of insertion of the fore- and hind-limbs).

Females with large-sized yolked follicles or oviductal eggs were transported to our laboratory in Wuzhishan, Hainan, where 6–8 individuals were housed together in 800 × 500 × 400 mm (length × width × height) communal indoor cages. The cages contained a substrate of moist soil (~100 mm depth) with litter layers and pieces of clay tiles provided as shelter and basking sites. Thermoregulatory opportunities were provided during daytime hours (07:00–19:00 h) by a 100 W full-spectrum lamp (EuroZoo, Germany); overnight temperatures varied from 21–28°C. Mealworms (*Tenebrio molitor*), house crickets (*Achetus domestica*) and field-captured grasshoppers dusted with multivitamins and minerals, and water were provided daily so that excess food was always available in the communal cages.

We checked the cages at least three times daily for

newborns after the first female gave birth, so that neonates were always collected, weighed, and measured less than 6 h post-parturition. Females were isolated from each other using dividers that created 400 × 300 × 400 mm chambers in the case that they gave birth during the same time period in the same cage, with none of them isolated for more than 48 h. Postpartum females were weighed and measured, and were then released at their point of capture, together with their offspring. Relative litter mass was calculated by dividing litter mass by postpartum body mass.

We used Statistica 6.0 (StatSoft, Tulsa, USA) to analyze data. Data were tested for normality using Kolmogorov-Smirnov tests, and for homogeneity of variances using Bartlett's test. Percentage data were arcsine transformed to satisfy the assumptions for parametric tests. We used independent-sample *t*-test to examine differences in SVL between male and female adults. We used linear regression analysis to examine whether a trait was dependent on body size. We used one-way analysis of covariance (ANCOVA) to examine whether the rates at which HL, HW, and AL increased with SVL differed between the sexes. We calculated regression residuals of HL, HW, and AL against SVL, and then used one-way analysis of variance (ANOVA) to examine whether HL, HW, and AL differed between the sexes. We used one-way ANCOVA with female SVL (for litter size, neonate mass, and litter mass) or postpartum body mass (for relative clutch mass) as the covariate to determine whether the examined reproductive variables differed among years and among females with different litter sizes. Partial correlation analysis was done to examine whether there was a trade-off between size and number of offspring while holding female SVL constant. Multiple comparisons were performed when necessary using Tukey's test. Descriptive statistics were presented as mean ± SE and range, and the significance level was set at α = 0.05.

3. Results

The range of SVL in adult males was 86–121 mm (106.8 ± 0.3 mm, n = 568), and in adult females was 86–125 mm (103.9 ± 0.2 mm, n = 1615) (Figure 1). Males were larger than females by an average of 3 mm SVL, and the difference was significant (t = 9.44, df = 2181, P < 0.0001). Based on a comparison of the largest 1/3 of males (n = 189) and females (n = 538), males also attained a larger mean body size (112.8 ± 0.2 mm SVL) than females (110.5 ± 0.1 mm; t = 10.62, df = 725, P <
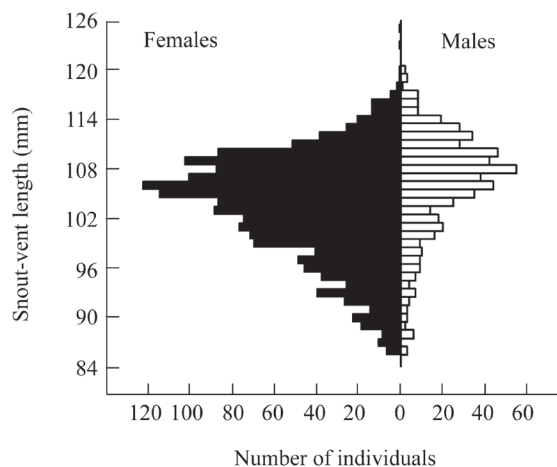

Figure 1 Size distribution of sexually mature female (n = 1615) and male (n = 568) *Eutropis multifasciata*.

0.0001). The rates at which HL and HW increased with SVL were both greater in adult males (ANCOVA; both P < 0.0001), whereas the rates at which AL increased with SVL did not differ between the sexes (ANCOVA; $F_{1, 763}$ = 1.37, P = 0.242) (Figure 2). HL ($F_{1, 765}$ = 669.90), HW ($F_{1, 765}$ = 530.79), and AL ($F_{1, 765}$ = 112.71) relative to SVL differed between the sexes (ANOVA on arcsine-transformed data; all P < 0.0001). Head size relative to SVL was greater in males, whereas AL relative to SVL was greater in females (Table 1).

The smallest reproductive female in our sample was 86 mm SVL. Females produced 2–9 offspring per litter (Table 2) and most gave birth between March and August. Year-to-year variation in litter size, neonate mass and litter mass was not significant (ANCOVA with female SVL as the covariate; all P > 0.668). Pooling data for different years, we found that litter size ($F_{1, 475}$ = 74.11, P < 0.0001), neonate mass ($F_{1, 475}$ = 29.64, P < 0.0001),

Table 1 Morphological traits of adult *Eutropis multifasciata*. Data are expressed as mean ± SE and range.

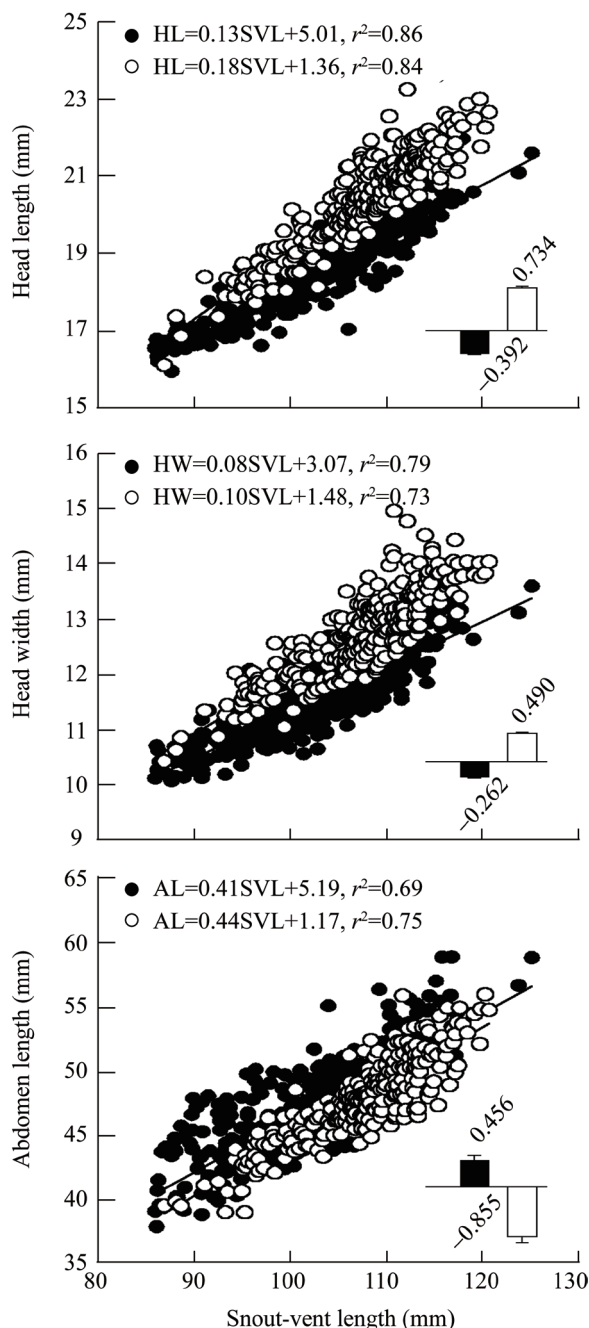|	Females	Males
n	500	267
Snout−vent length (mm)	103.5 ± 0.3	107.4 ± 0.4
	85.9−125.2	86.8−120.7
Head length (mm)	18.6 ± 0.05	20.4 ± 0.08
	16.0−22.0	16.1−23.3
Head width (mm)	11.6 ± 0.03	12.7 ± 0.05
	10.1−13.6	10.4−15.0
Abdomen length (mm)	47.7 ± 0.2	47.9 ± 0.2
	37.9−58.9	39.1−56.0
Relative head length,	18.0 ± 0.02	19.0 ± 0.03
HL/SVL (%)	16.1−19.7	17.6−20.7
Relative head width,	11.2 ± 0.02	11.9 ± 0.02
HW/SVL (%)	10.3−12.6	11.1−13.5
Relative abdomen length,	46.1 ± 0.1	44.6 ± 0.1
AL/SVL (%)	41.4−53.3	41.0−50.0

Figure 2 Linear regressions of head length, head width, and abdomen length on snout-vent length for adult *Eutropis multifasciata*. Females: Solid dots and bars; Males: Open dots and bars. The regression equations and coefficients are given in the figure. Mean values (numbers in the small plots) and +/–SE for regression residuals of the three sexually dimorphic traits against snout-vent length are also given in the figure.

and litter mass ($F_{1, 475}$ = 122.74, P < 0.0001) were all positively related to female SVL (Figure 3). Females with different litter sizes differed in SVL, neonate mass, litter mass and relative litter mass, but not in postpartum body mass (Table 2). At any given litter size, relative litter mass was negatively related to female SVL (linear regression

analysis; P < 0.04 in all cases). Pooling data for females with different litter sizes, we found once again that relative clutch mass was negatively related to SVL (linear regression analysis; $F_{1,475}$ = 12.62, P < 0.001) (Figure 4). Neonate mass was negatively correlated with litter size when holding female SVL constant (r = −0.14, t = 3.01, df = 474, P < 0.003).
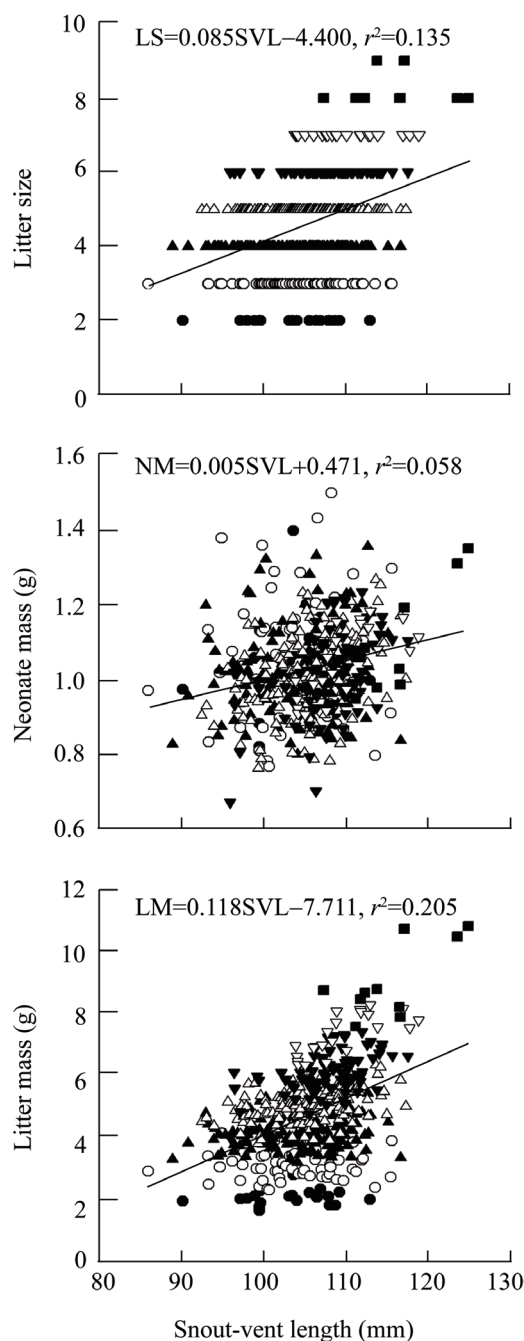

Figure 3 Linear regressions of litter size, neonate mass and litter mass against snout-vent length of female *Eutropis multifasciata*. ●, ○, ▲, △, ▼, ▽ and ■ represent females producing two, three, four, five, six, seven and 8–9 offspring per litter, respectively. The regression equations and coefficients, calculated after pooling data for females with different litter sizes, are given in the figure.

Table 2 Reproductive characteristics of female *Eutropis multifasciata* with different litter sizes. Data are expressed as mean ± SE and range. *F*-values of one-way ANOVA (for female SVL) or one-way ANCOVA (using female SVL for postpartum body mass, neonate mass and litter mass, and postpartum body mass for relative litter mass) are indicated. Means with different superscripts differed significantly (Tukey's test, α = 0.05). F_2, F_3, F_4, F_5, F_6, F_7 and F_{8-9} represent females producing two, three, four, five, six, seven and 8–9 offspring per litter, respectively.

	Litter size							Statistical analyses and the results of Tukey's multiple comparisons
	Two offspring	Three offspring	Four offspring	Five offspring	Six offspring	Seven offspring	8–9 offspring	
n	22	65	140	135	84	21	10	
Snout-vent length (mm)	103.1 ± 1.1	103.8 ± 0.7	104.3 ± 0.5	105.4 ± 0.4	107.9 ± 0.5	109.8 ± 1.0	115.7 ± 1.7	$F_{6, 470} = 15.26$, $P < 0.0001$
	90.2–113.1	85.9–115.8	88.5–116.9	92.4–117.5	96.0–117.7	104.0–119.1	107.4–125.2	$F_2{}^c$, $F_3{}^c$, $F_4{}^c$, $F_5{}^c$, $F_6{}^b$, $F_7{}^b$, $F_{8-9}{}^a$
Postpartum body mass (g)	26.0 ± 1.1	25.4 ± 0.7	25.4 ± 0.4	26.5 ± 0.4	28.1 ± 0.5	30.7 ± 1.3	34.8 ± 1.7	$F_{6, 469} = 1.22$, $P = 0.295$
	16.1–34.9	13.3–40.3	12.8–38.4	16.7–39.2	16.3–40.3	18.8–43.2	26.4–41.4	
Neonate mass (g)	1.03 ± 0.03	1.06 ± 0.02	1.03 ± 0.01	1.01 ± 0.01	1.01 ± 0.01	1.05 ± 0.02	1.10 ± 0.04	$F_{6, 469} = 2.73$, $P = 0.013$
	0.82–1.40	0.77–1.50	0.80–1.36	0.76–1.27	0.67–1.24	0.91–1.19	0.95–1.35	$F_2{}^{ab}$, $F_3{}^a$, $F_4{}^{ab}$, $F_5{}^b$, $F_6{}^b$, $F_7{}^{ab}$, $F_{8-9}{}^{ab}$
Litter mass (g)	2.07 ± 0.05	3.17 ± 0.06	4.11 ± 0.04	5.05 ± 0.05	6.07 ± 0.07	7.33 ± 0.15	9.06 ± 0.38	$F_{6, 469} = 200.91$, $P < 0.0001$
	1.64–2.80	2.30–4.51	3.20–5.43	3.81–6.34	4.02–7.42	6.34–8.30	7.59–10.83	$F_2{}^g$, $F_3{}^f$, $F_4{}^e$, $F_5{}^d$, $F_6{}^c$, $F_7{}^b$, $F_{8-9}{}^a$
Relative clutch mass	0.08 ± 0.004	0.13 ± 0.004	0.17 ± 0.003	0.20 ± 0.003	0.22 ± 0.004	0.25 ± 0.009	0.26 ± 0.01	$F_{6, 469} = 195.49$, $P < 0.0001$
	0.05–0.12	0.07–0.25	0.10–0.29	0.11–0.31	0.14–0.33	0.18–0.35	0.19–0.33	$F_2{}^d$, $F_3{}^c$, $F_4{}^b$, $F_5{}^a$, $F_6{}^a$, $F_7{}^a$, $F_{8-9}{}^{ab}$

4. Discussion

Far more individuals were measured for SVL in the present study (1615 adult females and 568 adult males) than in the previous study (189 adult females and 124 adult males; Ji *et al.*, 2006), thus allowing more accurate determination of sexual size dimorphism (SSD). Adult males were larger than adult females in mean SVL, which confirms the previous conclusion that *Eut. multifasciata* displays male-biased SSD (Ji *et al.*, 2006). In the present study, 14 males were larger than the largest male reported previously (117 mm SVL; Ji *et al.*, 2006), and 25 females were larger than the largest female reported previously (116 mm SVL; Ji *et al.*, 2006). Adult males were larger than adult females by an average of 3 mm SVL in the present study, and by an average of 5 mm SVL in the previous study (Ji *et al.*, 2006). Adult males measured in the two studies had almost the same mean SVL (~107 mm in both studies), whereas adult females in the present study were larger than those measured previously by an average of 2 mm SVL. Thus, less pronounced SSD detected in the present study primarily resulted from a greater mean SVL in adult females.

Adult males had larger heads and were shorter in AL than females of the same SVL, which is consistent with the results reported previously for *Eut. multifasciata* (Ji *et al.*, 2006; Sun *et al.*, 2012a), and also for numerous other lizard species (Braña, 1996; Olsson *et al.*, 2002; Cox *et al.*, 2003; Kratochvíl *et al.*, 2003; Pincheira-Donoso and Tregenza, 2011). The rate at which HL increased with SVL was greater in adult males (Figure 2), which is consistent with the result reported previously for *Eut. multifasciata* (Ji *et al.*, 2006). The rate at which HW increased with SVL was greater in adult males (Figure 2) as opposed to the previous conclusion that the rate does not differ between the sexes (Ji *et al.*, 2006).

Females produced up to nine offspring per litter as opposed to the previously reported 2–7 (Ji *et al.*, 2006). Most females gave birth between March and August, a time period approximately four months longer than that (May– June) reported previously for the same population (Ji *et al.*, 2006). Females with a higher fecundity (litter size) tended to produce smaller offspring as opposed to the previous conclusion that female *Eut. multifasciata* do not tradeoff offspring size against number (Ji *et al.*, 2006). Neonate mass varied from 0.67−1.50 g, a range wider than that (0.77−1.40 g) reported previously for the same population (Ji *et al.*, 2006). These differences largely resulted from the difference in the number of females sampled [previous study: n = 35 (Ji *et al.*, 2006);
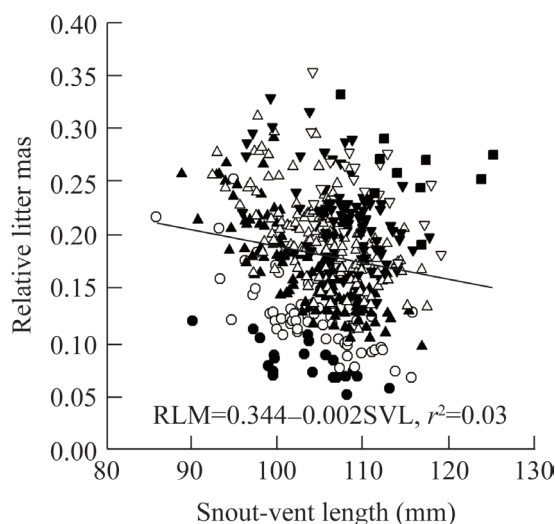
Figure 4 Relationship between relative clutch mass and snout-vent length of female *Eutropis multifasciata* with different litter sizes. ●, ○, ▲, △, ▼, ▽ and ■ represent females producing two, three, four, five, six, seven and 8–9 offspring per litter, respectively. The regression equation and coefficient, calculated after pooling data for females with different litter sizes, are given in the figure.

present study: n = 477 (Table 2)]. Mean values for litter size (4.6 *vs.* 4.1), neonate mass (1.03 *vs.* 1.00 g) and litter mass (4.75 *vs.* 4.10 g) were greater in the present study (Table 2) than in the previous one (Ji *et al.*, 2006). These differences primarily resulted from the difference in mean maternal SVL between the present (105.6 mm; Table 2) and previous (100.5 mm; Ji *et al.*, 2006) studies, as none of these three reproductive variables differed between the two studies after accounting for maternal SVL (ANCOVA; $P > 0.672$ in all cases). Year-to-year variation in litter size, neonate mass and litter mass was not found in the present study. Thus, taken together, we conclude that in *Eut. multifasciata* litter size, neonate mass and litter mass remain almost unchanged among years.

Litter size has been documented in several "*Mabuya*" skinks with viviparous reproduction (Table 3), but in none of these species except *Eut. multifasciata* (Ji *et al.*, 2006) have neonate mass and litter mass been available. The mean litter size of *Eut. multifasciata* was much smaller than that (13.2) reported for *Euprepis capensis*, but was similar to the values (4.6−5.8) reported for other viviparous "*Mabuya*" skinks so far studied (Table 3). In the present study, larger females generally produced more and larger offspring, and thus heavier litters than did smaller ones (Table 2, Figure 3). This confirms the previous conclusion that maternal body size is an important determinant of reproductive output in *Eut. multifasciata* (Ji *et al.*, 2006). Of the three reproductive variables (litter size, neonate mass and litter mass)

relating to reproductive output, litter mass was most tightly related to female body size, as revealed by the fact that female SVL explained a greater proportion of variation in litter mass (20.8%) than litter size (13.5%) and neonate mass (5.6%) (Figure 3). Although females with a higher fecundity tended to produce smaller offspring, larger females with a greater amount of energy to invest were more likely to vary the number but not the size of their offspring, as revealed by the fact that female SVL explained a greater proportion of variation in litter size than neonate mass.

In the present study, females differing in fecundity and reproductive output did not differ from each other in postpartum body mass, and relative clutch mass was negatively related to female body size. These results suggest that smaller females can produce relatively heavier litters without a concomitant reduction in postpartum body condition. The likely way through which smaller females produce relatively heavier clutches while keeping postpartum body condition constant is to divert energy that would be otherwise allocated towards growth to reproduction. Presumably, as in the Reevese's Butterfly Lizard *Leiolepis reevesii* (Du *et al.*, 2011) and the Oriental Leaf-Toed Gecko *Hemidactylus bowringii* (Xu and Ji, 2007) where a negative correlation between relative clutch mass and female size better explains female-smaller SSD in the two oviparous species, the preferential allocation of energy to the production of offspring rather than growth in sexually mature females provides one explanation for female-smaller SSD in *Eut. multifasciata*. It is worth noting that, though statistically significant, only 3% of variation in relative reproductive output (relative litter mass) could be explained by female SVL in *Eut. multifasciata* (Figure 4), a proportion much lower than those reported for *L. reevesii* (13%; Du *et al.*, 2011) and *H. bowringii* (17%; Xu and Ji, 2007).

In summary, our data confirm that *Eut. multifasciata*

Table 3 Mean and range for litter size of viviparous "*Mabuya*" skinks so far studied.

Species	Mean	Range	References
Euprepis capensis	13.2	8–20	Flemming, 1994
Eup. striata punctatissima	4.8	1–10	Patterson, 1990
Eup. striata striata	5.6	2–12	Patterson, 1990
Eutropis multifasciata	4.6	2–9	This study
Mabuya bistriata	4.7	2–9	Vitt and Blackburn, 1991
M. caissara	5.4	2–9	Simbotwe, 1980
M. frenata	4.9	2–8	Vrcibradic and Rocha, 1998
M. heathi	5.0	2–9	Vitt and Blackburn, 1983
M.mabouya	5.8	4–7	Ramírez-Pinilla *et al.*, 2002

adults are sexually dimorphic in body size and shape, and specifically, three aspects differing between the sexes are the greater mean SVL in males, the greater relative head size in males and the greater relative abdomen size in females. Our data also confirm that maternal size is the main determinant of reproductive investment in *Eut. multifasciata*, with larger females generally producing more, as well as larger, offspring. Litter size, neonate mass and litter mass remain remarkably constant among years. Of these three reproductive variables, litter mass is most tightly related to female body size. Contrary to the previous conclusion that the rate at which HW increases with SVL does not differ between the sexes, our data show that the rate is greater in adult males. Contrary to the previous conclusion that female *Eut. multifasciata* do not tradeoff offspring size against number, our data show that females with a higher fecundity tend to produce smaller offspring.

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